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Neutrophil Enzymes in the Lung: Regulation of Neutrophil Elastase[1–3]

STEWART A. LONKY and JOHN McCARREN

Introduction

In 1963, Laurell and Erickson (1) recognized a higher than expected incidence of lung disease in patients with an inherited deficiency of alpha 1 protease inhibitor (A1Pi). Since that time, Janoff and Scherer (2) have demonstrated that the granules of human neutrophils contain proteolytic enzymes with activity at neutral pH, whereas a number of other investigators have shown that the intratracheal administration (3, 4) of neutrophil elastase (NE) induces experimental emphysema in laboratory animals. These data have prompted the notion that a relative excess of NE in the lung leads to elastic tissue disruption and pulmonary emphysema. The fact that the majority of patients with emphysema have normal serum levels of A1Pi suggests that if such a protease pathogenesis theory is correct, periods of imbalance between elastolytic enzymes and elastolytic inhibitors must exist in the lung. Accordingly, a number of laboratories have sought to identify factors that either diminish lung antiprotease activity or increase NE activity in the lung. In this report we shall address our attention to the data regarding factors that increase NE activity either intracellularly or after the enzyme has been released from neutrophil granules.

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The Intracellular Regulation of Neutrophil Elastase

Synthesis of the Enzyme

In the mature neutrophil, elastolytic enzyme activity is found in the azurophilic granules (5–7). These granules are formed only during the promyelocyte stage of neutrophil development (8), and are subsequently distributed to daughter cells following cellular mitosis. Mitotic divisions cease after the myelocyte stage, and further cell maturation is accompanied by a decrease in cellular organelles essential for protein synthesis. Specifically, the nuclear chromatin becomes coarsely clumped, nucleoli disappear, and there is a dramatic reduction in free ribosomes and rough endoplasmic reticula (8). These findings suggest that the elastase content of the mature neutrophil is determined during the early stages of granulopoiesis. However, Granelli-Piperno and associates (9) have shown that more than 60% of mature human neutrophils isolated from peripheral blood retain the capacity to synthesize RNA and other proteins. In addition, both neutrophil biosynthetic activity and the release of newly synthesized macromolecules are influenced by a variety of chemicals.

Clark and coworkers (10), using cytochemical techniques, found evidence for the existence of elastase-like enzymes within the nuclear envelope, endoplasmic reticulum, golgi complex, and some granules of circulating neutrophils. Thus it appears that the circulating neutrophil is capable of NE synthesis. Whether factors that increase NE activity act at the level of neutrophil precursors or on mature neutrophils is yet to be determined.

Clinical Correlates of Increased Neutrophil Elastase

In an attempt to establish a relationship between protease-antiprotease imbalance and clinical disease, a number of investigators have measured the levels of NE in patients with chronic obstructive pulmonary disease (COPD), and compared these with levels measured in normal subjects. Goldston and colleagues (11) reported there are increased degrees of neutrophil elastase-like esterolytic activity in patients with COPD and a variety of Pi phenotypes. Lam and coworkers (12) demonstrated that higher than normal levels of NE constitute a significant risk factor for the development of COPD in PiMZ patients, and Rodriguez and associates (13) found that NE levels are increased in COPD patients with PiMM phenotype. Although Kramps and coworkers (14) failed to find any correlation between NE levels and the presence of lung disease in PiZZ patients, these investigators did note that PiMM patients with COPD had higher than normal levels of NE. These studies suggest that high levels of NE represent a potential risk factor for the development of COPD, and that other factors, such as the status of lung antiprotease activity, also play an important role in determining the presence or absence of clinical disease.

Attempts to correlate the level of NE in peripheral blood with disease severity have not been uniformly successful. Although Kidokoro and coworkers (15) found more lung hyperinflation and more severe impairment of carbon monoxide diffusing capacity in PiZZ patients with higher NE levels, Rodriguez and associates (13) failed to find any such correlation in COPD patients with the PiMM or PiMZ phenotype. Additional, well controlled studies are necessary before this issue can be resolved, but it is reasonable to believe that NE levels are one of the variables that dictate the degree of connective tissue disruption in COPD.

Relatively little information is available regarding the factors that regulate the level of NE in circulating or lung neutrophils. It has been demonstrated (13, 15) that these levels are relatively stable over a period of 6 months to 1 yr in patients with uncomplicated disease, and it appears that these NE levels are not dependent on age, sex, or Pi type (11, 14). Although it has been suggested that intracellular NE levels might be genetically determined (16), there are preliminary data indicating that both transient and more sustained increases in NE activity can be acquired. Cigarette smokers have increased levels of NE when compared with matched control subjects (12), and both patients (13) and laboratory animals (17) with pneumococcal pneumonia have transient elevations in NE activity.

Neutrophil Elastase and Pneumococcal Pneumonia: Protease Antiprotease Homeostasis

Design of the study. The longstanding association between infection and COPD prompted us to investigate the relationship between NE levels and pulmonary infection. Pulmonary infection with the Type III pneumococcus was selected as an experimental model because infection with this organism induces a brisk-neutrophil response in the lung. Furthermore, nonlethal lobar pneumonia can be induced in dogs by intratracheal inoculation of this organism suspended in starch broth (18, 19). Conditioned dogs were inoculated with either normal saline (control dogs) or Type III pneumococcus (pneumonia dogs), and the inoculum was directed to the right lower lobe under fluoroscopic guidance. Serial roentgenographs, CBCs, and temperature measurements confirmed that a lobar pneumonia was induced in 11 of 14 dogs, and cultures of bronchial and lung aspirate confirmed that the pneumococcus was the bacterial agent. No control animal developed pneumonia. Peripheral blood granulocytes were collected from 50 ml of blood (17), and elastase-like esterase activity against N-t-Boc alanine-p-nitrophenyl ester (NBA) was measured (20). In addition, peripheral blood antiprotease activity was determined (21). Bronchoalveolar lavage was performed via a fiberoptic bronchoscope wedged in the uninoculated left lower lobe, and bronchoalveolar lavage fluid (BALF) and cells were collected.

Time course and magnitude of NE response. Within 24 hours, the 9 dogs surviving pneumonia induction demonstrated a statistically significant increase in circulating neutrophils (6.5 ± 0.9 to 14.9 ± 2.1 × 10^3 cell m/ml), and an increase in the percentage of circulating immature neutrophils from 4% to 24%. Although the mean circulating neutrophil count increased somewhat in 5 control animals, this increase was not statistically significant. Within 24 hours of pneumococcal inoculation, infected dogs showed a 40 to 60% increase in NBA esterase levels compared with preinfection levels, and this increase in circulating NBA esterase activity persisted for 48 h (figure 1). A similar increase in NBA esterase activity was found in neutrophils derived from the BALF of infected dogs, indicating that the lung and its connective tissue substrate is exposed to this increased enzymatic potential. Preliminary data from humans with acute bacterial pneumonia support this finding in dogs, revealing transient, statistically significant increases in circulating NE levels during active infection (13).

It is of interest to note that uninfected animals also experienced an increase in circulating neutrophil elastase-like esterase activity, but that this increase occurred more slowly, reaching statistical significance only on the fourth post-infection day (figure 1). Of these 5 control animals, 3 had bronchoalveolar lavage performed daily, whereas the other 2 had lavage performed only once (on the day of inoculation).

A further analysis of data from these animals revealed that the increase in NBA esterase activity shown in figure 1 for con-

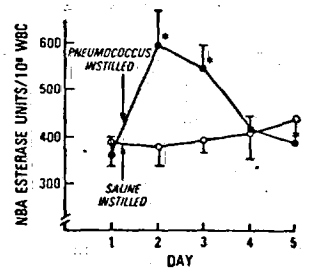

Fig. 1. Changes in mean circulating NBA esterase activity in 9 dogs intratracheal pneumococcus (*closed circles*) and in 5 dogs receiving intratracheal saline (*open circles*). Asterisks signify that NBA esterase levels are higher in pneumonia dogs with $p < 0.02$ (Student's *t* test).

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trol dogs can be attributed to the lavage process because unlavaged control subjects had only a 2 to 4% increase in NBA esterase activity, whereas lavaged animals experienced a 14.5% increase (17). No significant increase in the circulating neutrophil count was detected in the 3 control dogs that underwent bronchoalveolar lavage, but each of these 3 animals experienced a brisk influx of neutrophils to the lung. Studies in monkeys and in a large number of dogs have verified that either bronchoscopy or bronchoalveolar lavage is associated with a pulmonary neutrophil response (22). These studies also reported an increase in peripheral blood neutrophils and an increase in immature band forms following bronchoscopy or lavage. Therefore, in addition to pneumococcal pneumonia, bronchoscopy and repeated bronchoalveolar lavage can expose the lungs to increased levels of NE.

Time course and magnitude of antiprotease response. The data described thus far suggest that pulmonary infection can result in an increased risk for elastolysis. The fact that pneumococcal pneumonia is not usually followed by impaired lung elasticity prompted us to measure the protease inhibitory activity in the peripheral blood and lungs of infected and noninfected animals. We found that the serum trypsin inhibitory capacity (TIC) and the serum NBA esterase inhibitory activity (EIC) increased 15% in dogs with pneumonia, and remained elevated for 48 h (figure 2). Furthermore, we found a striking increase in BALF NBA esterase inhibitory activity following infection, with the mean inhibitory activity increasing from 776 ± 200 to 1400 ± 190 NBA units inhibited/mg lavage protein (mean ± SE).

Subsequent studies have shown that this increase in lavage EIC is due to an increase in A1Pi. Thus it appears that the lung is protected from proteolytic attack during pneumococcal lung infection by a simultaneous increase in peripheral blood and lung protease inhibitory activities. Control animals did not demonstrate any statistically significant changes in either peripheral blood or lung lavage antiprotease activity, even when control animals undergoing bronchoalveolar lavage were considered separately. These data suggest that protease and antiprotease activities following bronchoscopy and bronchoalveolar lavage may be modulated by independent factors.

Extracellular Regulation of Neutrophil Elastase

Once the neutrophil has migrated to the lung, a variety of stimuli can trigger the extracellular release of granular contents. Models for the mechanism of extracellular granule release have been reviewed elsewhere (23), and will not be reconsidered here. For NE, the available data indicate that it is released from neutrophil granules as an active enzyme, free to act on any of its substrates. Whether NE actually attacks a particular substrate, however, depends on the balance struck between factors that either enhance or diminish NE activity. Such factors include the location of enzyme release, the presence or absence of enzyme inhibitors, and the presence of various molecules that can enhance NE activity.

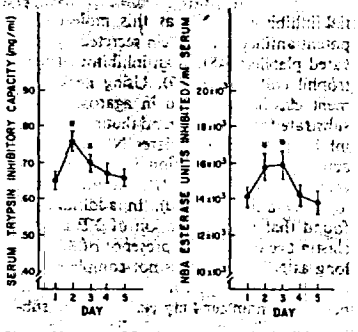

Fig. 2. Mean ± SE of serum trypsin inhibitory capacity (*left*) and serum NBA esterase inhibitory capacity (*right*) in 9 dogs inoculated with pneumococcus. All animals inoculated on Day 1. Asterisks signify levels significantly greater than preinfection levels with $p < 0.05$ (Student's *t* test).

Location of Enzyme Release

In the lung, NE can be released into any of the 3 major compartments: vascular, interstitial, or bronchoalveolar. Potential substrates for this enzyme exist in each compartment because NE is a proteolytic as well as an elastolytic enzyme. NE will attack a variety of proteins, including complement (24), components of the clotting system (25), immunoglobulins (25), kinins (25), collagen (26), basement membranes (27, 28), and elastin (3, 29). It is, however, the action of NE against lung elastic tissue and the induction of an emphysema-like lesion (3, 5) that has been most extensively studied, and which will be reviewed here.

Because elastin is found in the lung interstitium, NE release into this compartment would be expected to result in the greatest amount of elastic tissue damage. Although such interstitial release of NE has not been directly measured, there is evidence that such release takes place. Wright and Gallin (30) have shown that the chemotaxis of PMNs is associated with the leakage of various enzymes, and the movement of PMNs through the interstitial space can be expected to result in small amounts of NE release. In addition, a variety of inflammatory reactions in the lung are associated with the interstitial accumulation of PMNs. Some of these reactions are due to agents that are capable of causing PMN degranulation (23), and NE release is inevitable under these circumstances.

Because both the vascular and bronchoalveolar compartments contain protease inhibitors, NE released into these compartments is subject to inactivation. As the vascular compartment contains a greater amount of inhibitory protein, one would expect that lung elastolytic damage would be less if NE were introduced via this route. Animal studies have borne out this hypothesis, demonstrating that although experimental emphysema can be induced by the intravascular administration of elastolytic enzymes (31), enzyme administered intratracheally is more potent against lung elastin (32).

NE released into the bronchoalveolar compartment is subject to the effects of various inhaled gases, aspirated material, and airborne particulate matter. Any of these factors can alter the physical-chemical environment in which the enzyme will act, resulting in a change in enzyme activity. To date, there have been few studies directed at measuring such changes in NE activity *in vivo* but we can predict some expected effects by considering the available *in vitro* data. The elastolytic activity of NE is decreased when the pH is decreased below 7.4 and approaches zero at pH = 5.0 (33). It is intriguing to hypothesize that the inhalation of gases such as ozone, oxides of nitrogen, or chlorine, which can diminish the pH of bronchial secretions, might decrease NE activity. In addition, the aspiration of acid stomach contents might inhibit the action of free bronchoalveolar NE. Conversely, the alkalinization of bronchial secretions might result in enzyme stimulation because NE reaches peak elastolytic activity at pH 8.8 (33). Changes in the ionic strength of bronchial secretions, induced by the transudation of fluid or the inhalation of various solutions, also might affect NE activity. Increases in ionic strength can result in NE stimulation against both elastin (34) and synthetic substrates (35), whereas decreases in ionic strength can diminish NE elastolytic activity.

Presence of Protease Inhibitors

In each of the lung compartments are protease inhibitors that can neutralize NE activity. As previously stated, the major serum and bronchoalveolar fluid inhibitor of NE is A1Pi (36). The mechanism of action of this protease inhibitor has been described (37), and the functional regulation of A1Pi is the subject of another report in this symposium. A1Pi is made predominantly in the liver (38). More recent data have demonstrated that the alveolar macrophage is also capable of A1Pi synthesis (39), although it is likely that macrophage-derived A1Pi constitutes only a fraction of BALF A1Pi. In the serum, A1Pi is an acute phase reactant with increases in levels occurring in response to lobar pneumonia, pregnancy, birth control pills, malignant tumors, severe burns, and typhoid vaccine (38). In the bronchoalveolar compartment, A1Pi levels are increased following infection with the pneumococcus (17), and in the

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bronchoalveolar lavage fluid of smokers (40). Other factors that might increase bronchoalveolar levels of this protease inhibitor are still to be identified. Because this compartment is exposed to inhaled and aspirated material, it is likely that the level or activity of bronchoalveolar A1Pi is more susceptible to change than serum levels or activity.

In addition to A1Pi, both the vascular and bronchoalveolar compartments contain alpha 2 macroglobulin (α2M), a large (MW = 725,000) protein that can inhibit NE elastolytic activity (41). Whereas A1Pi completely inhibits NE activity, experimental data have shown that NE activity against elastin is incompletely inhibited by α2M (41). The residual activity of α2M-NE complexes against elastin may play an important role in patients who are genetically or functionally deficient in A1Pi (41, 42). Two other NE inhibitors have been identified in the bronchoalveolar compartment, neither of which has been fully characterized. The first of these is a low molecular weight (MW = 14,000) acid-stable inhibitor found in purulent sputum (43) and bronchoalveolar lavage fluid (44). The second bronchoalveolar inhibitor is a high molecular weight (MW = 80,000) inhibitor found in bronchoalveolar lavage fluid of dogs and humans (45). The role of these inhibitors *in vivo* is yet to be ascertained.

Factors That Stimulate NE Activity

Substrate considerations. Elastin is a cross-linked structural protein containing an unusually high percentage of nonpolar amino acids (46). These qualities render elastin insoluble in aqueous systems. Because of this, multiple synthetic substrates have been developed that allow for the rapid spectrophotometric determination of elastase activity. The most widely used of these substrates are N-t-Boc-L-alanine-p-nitrophenyl ester (NBA) and succinyl-trialanine-p nitroanilide (Suc-$(Ala)_3$-NA), each of which has been used as substrate for pancreatic elastase or NE (18, 47). It must be remembered, however, that these synthetic substrates measure the esterolytic activity of NE, and although this esterolytic activity parallels the elastolytic activity of the enzyme, factors that influence the esterase activity of NE may have little or no effect on the elastolytic activity of the enzyme, and vice versa. This fact is well demonstrated by reviewing the effects of α2M on NE activity. Whereas NE elastolytic activity is almost completely abolished by α2M, the activity of NE against Suc-$(Ala)_3$-NA is markedly increased. Because it is elastin that is the substrate in lung parenchyma, we have focused our attention on factors that stimulate NE activity against either lung elastin or elastin purified from lung tissue or bovine ligamentum nuchae.

Stimulation of NE by platelet factor 4. We are initially interested in PF_4 as a potential inhibitor of NE as this molecule, a potent antiheparin protein secreted by activated platelets (48), is an inhibitor of neutrophil collagenase (49). Using neck ligament elastin suspended in agarose as the substrate for NE, we found that rather than inhibit NE, PF_4 stimulates NE elastolytic activity. This stimulation is seen at concentrations of PF_4 and NE that are physiologically attainable (50). In addition, we found that this stimulation of NE against elastin occurred in the presence of A1Pi as long as NE activity was not completely inhibited. Studies performed using radiolabeled (51) hamster lung elastin as the substrate confirmed these findings, demonstrating a 50% increase in NE activity when the molar ratio of PF_4:NE was 4:1, and a doubling of NE activity when the ratio was 10:1 (figure 3). When a synthetic NE substrate such as NBA was used, we found no enhancement of NE esterolytic activity (figure 3). The proteolytic activity of NE against either casein or hemoglobin is only slightly increased at high molar ratio of PF_4:NE (figure 3). Thus the ability of PF_4 to stimulate NE is relatively specific for NE elastolytic activity.

To test whether PF_4 stimulated NE activity against lung elastin in situ, hamster lungs were inflated with solutions of buffer alone (phosphate buffered saline pH 7.4; PBS), human NE, PF_4 alone, or PF_4 and NE mixed at a molar ratio of PF_4:NE = 4:1. After a 2-hour incubation at 37° C, the lungs inflated with NE alone showed minimal alveolar hyperinflation. Pressure-volume studies revealed that the compliance of these "low dose" NE lungs was not different from the compliance of control lungs. The addition of PF_4 to the same amount of NE resulted in a more severe alveolar injury (figure 4) and a shift of the lung pressure-volume curve to the left (figure 5). Measurements of lung connective tissue content revealed that a significant decrease in measurable lung elastin occurred only in the animals treated with NE plus PF_4, and that collagen content was unaffected (51). Thus, PF_4 is capable of stimulating NE against lung elastin in situ, and such stimulation may result in connective tissue destruction by levels of NE that otherwise would be ineffective.

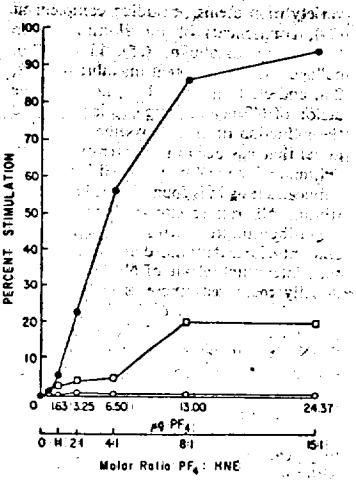

Fig. 3. Stimulation of human neutrophil elastase (HNE) by PF_4 against ^{3}H-lung elastin (*closed circles*), ^{125}I-casein (*open squares*), and N-t-boc-alanine-p-nitrophenyl ester (NBA; *open circles*). All experiments done with 5 μg HNE. 5 μg resulted in release of 1.9×10^3 cpm from ^{3}H elastin, and 1.2×10^3 cpm from ^{125}I casein. Incubations at 37° C for 3 h.

PF_4 as a model stimulant of NE activity; effects of other lysine-rich ligands. PF_4 is a basic protein at physiologic pH, and a study of the amino acid sequence of this protein reveals that its cationic nature is the result of a lysine-rich region near the carboxy-terminus of this 70 amino acid molecule

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(. . . Lys-Lys-Ile-Ile-Lys-Lys . . .) (52). We have found that upon mixing with NE, PF_4 is rapidly cleaved into at least 3 fragments (51). However, PF_4 which has been incubated with NE for 1 hour, still retains NE stimulatory activity, suggesting that one or more of the PF_4 fragments are capable of stimulating NE. Preliminary data have shown that some of the PF_4 fragments contain the lysine-rich carboxy terminal region and remain cationic. These data prompted us to study the effect of other lysine-rich molecules on NE activity.

To date we have studied various poly-L-lysines and histones that are relatively lysine-rich (histone V and histone IIIs; Sigma, St. Louis, MO). We found that poly-L-lysines with a polymer length greater than 4 lysines are capable of stimulating NE activity (table I), and that histone IIIs is also capable of such stimulation. This property is not found in other basic molecules because poly-L-arginine, α chymotrypsin, and trypsin fail to stimulate NE activity (table I).

Previous investigators have shown that elastin is slightly anionic in nature (53), and Gertler (54) has shown that basic ligands such as poly-L-lysine, poly-L-arginine, and pancreatic elastase bind to elastin in a non-specific, electrostatic manner. We expect that PF_4 and other lysine-rich ligands also bind to elastin electrostatically, and that this substrate binding plays an important role in NE stimulation. This theory is in disagreement with experimental observations for pancreatic elastase, which show that pancreatic elastase activity against elastin is wholly governed by the presence of charge complementarity between enzyme and substrate (55). However, for NE, electrostatic binding of enzyme to substrate is likely to be less important than for pancreatic elastase. Evidence that NE intereacts

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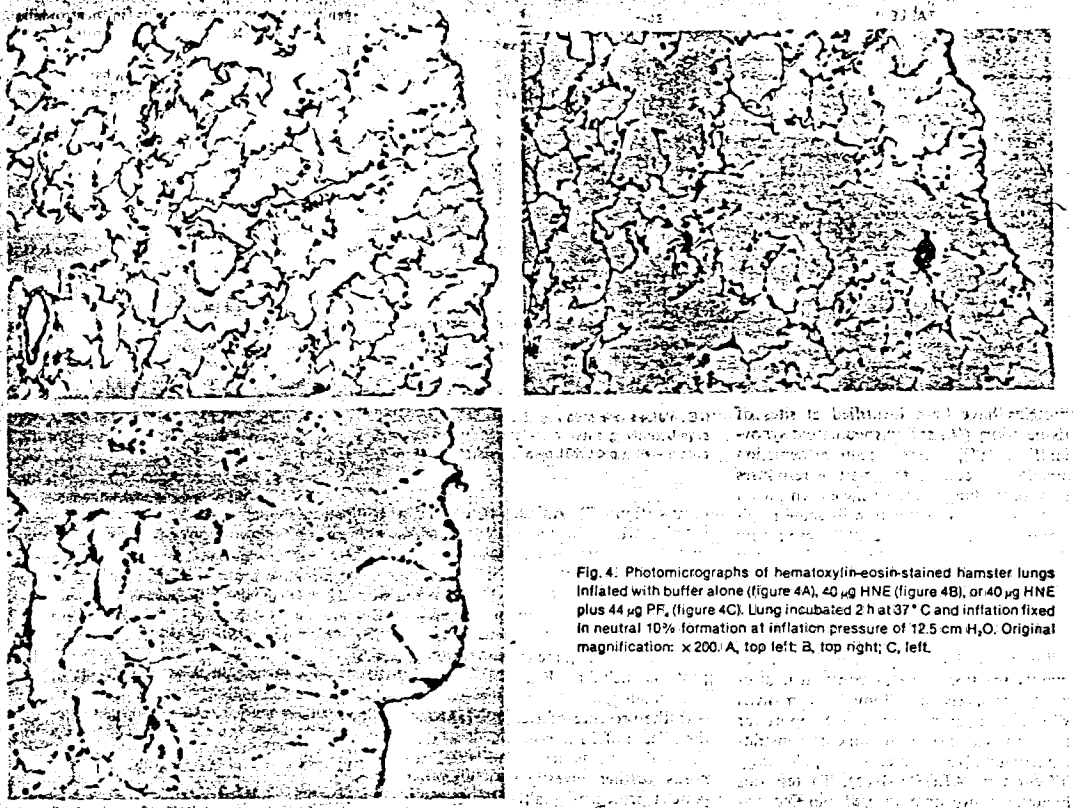

Fig. 4. Photomicrographs of hematoxylin-eosin-stained hamster lungs inflated with buffer alone (figure 4A), 40 μg HNE (figure 4B), or 40 μg HNE plus 44 μg PF_4 (figure 4C). Lung incubated 2 h at 37° C and inflation fixed in neutral 10% formation at inflation pressure of 12.5 cm H_2O. Original magnification: ×200. A, top left; B, top right; C, left.

with elastin via nonelectrostatic mechanisms comes from work by Boudier and coworkers (34) and Liestienne and Bieth (35), who have shown that NE elastolytic and esterolytic activity are increased in the presence of high ionic strength, a condition that rapidly inactivates the pancreatic enzyme (25, 33).

Support for the hypothesis that PF_4 stimulates NE by attaching to the substrate (substrate directed stimulation), rather than by a direct action of PF_4 on NE, comes from experiments in which PF_4 was preinfused into elastin-agarose plates and followed by the addition of NE (figure 6). Stimulation of NE results, and the degree of stimulation is somewhat greater than that seen when PF_4 and NE are mixed before addition to the elastin plate wells.

From these data we have theorized that PF_4 released into the lung, even in the absence of concomitant NE release, can be adsorbed onto elastin, resulting in a substrate that is more susceptible to attack by NE at a later time.

Platelets and PF_4 in the lung; the "inflammatory" platelet. The data outlined above take on increased significance when one considers the fact that platelets are a normal constituent of circulating blood, and that activated platelets can release PF_4 endogenously (56). Recent evidence has pointed to an additional role for platelets, that being a role in the inflammatory reaction. Several agents that initiate inflammatory reactions, such as urate crystals (57), immune aggregates (58), and bacterial lipopoly saccharides (59), can stimulate platelets and result in the release of platelet contents. In addition, platelets and platelet

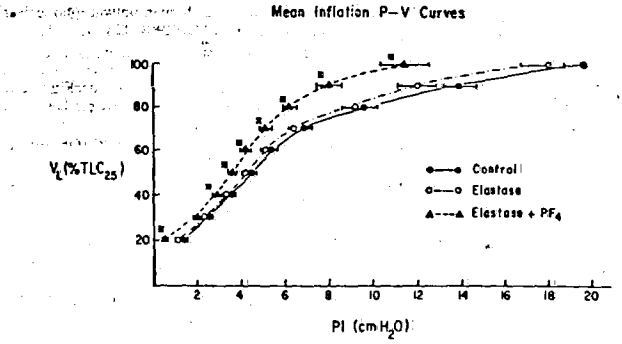

Fig. 5. Mean inflation PV curves for animals receiving buffer alone (●), 40 μg HNE (○), or 40 μg HNE plus 44 μg PF_4 (▲). Asterisks indicate data points significantly different from control values by analysis of variance. Mean deflation P-V curve (not shown) reveals similar results, with HNE + PF_4 curve significantly more compliant at lung volumes (V_L) from 50% to 100% of total lung capacity (TLC_{25}).

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TABLE 1
RELATIVE ELASTOLYTIC ACTIVITY OF NEUTROPHIL ELASTASE[a,b]

Ligand Tested	Molar Ratio Cationic Ligand:NE 2:1	4:1	10:1
Poly-L-lysine (30,000)	2.20	2.20	2.20
Poly-L-lysine (13,000)	1.70	1.90	2.0
PF_4	1.50	1.75	1.75
Histone IIIs	1.18	1.35	1.55
Poly-L-lysine (3,400)	1.30	1.40	1.45
Lysine (8-12)	1.05	1.10	1.25
Tetralysine	1.00	0.95	0.95
α chymotrypsin	1.00	0.90	0.90
Poly-L-arginine (13,900)	0.90	0.85	0.80

a. Activity of 5 μg NE alone against ^{3}H-elastin = 1.0.
b. All experiments done using 5 μg NE.

proteins have been identified at sites of tissue injury (60) and in rheumatoid synovial fluid (61), whereas platelet depletion ameliorates certain flammatory responses (56). In the lung, platelets have been shown to accumulate in patients with acute respiratory failure (62) or the adult respiratory distress syndrome (ARDS) (63). Thus, the platelet probably plays an important role in lung inflammation, and we investigated the possibility that lung PF_4 release could occur *in vivo*.

Because PMNs play a role in the inflammatory reaction, we reasoned that neutrophil products such as NE might be involved with a lung platelet response. Support for this notion comes from work demonstrating free lung NE in patients with pulmonary infection or ARDS (63-65). To test this hypothesis, we administered purified human NE intratracheally to rabbits with ^{111}Indium-labeled platelets circulating in their peripheral blood pool (66, 67). We found that within 60 min of NE inoculation, a significant increase in lung platelets occurs (figure 7). Animals inoculated with inactive NE or buffer alone do not show such lung platelet sequestration. In addition, measurement of plasma and bronchoalveolar lavage fluid PF_4 levels by radioimmunoassay revealed that platelets were activated in the NE-inoculated animals. These activated platelets secreted platelet proteins including PF_4. Thus pulmonary inflammatory reactions that are associated with the presence of free NE can result in the release of PF_4. Whether other lung injuries also are associated with PF_4 release needs further investigation because this platelet protein may alter the reactivity of lung elastin, creating an imbalance in the lung protease-antiprotease system.

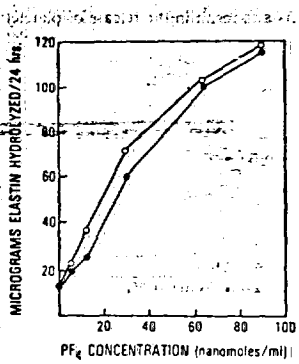

Fig. 6. Stimulation of human NE by PF_4 against elastin in elastin-agar plates. (Alphasin, Elastin Products, St. Louis, MO). Closed circles (●) show results when 16.6 nanomoles of NE preincubated with PF_4 and then added to plate. Open circles (○) show results when PF_4 is preinfused into elastin-agar, and NE added later.

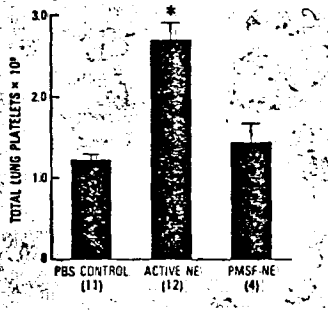

Fig. 7. Bar graph showing lung platelet counts 60 min after intratracheal inoculation of phosphate buffered saline (PBS), human neutrophil elastase (active NE), or human neutrophil elastase inactivated by phenyl methyl sulfonyl fluoride (PMSF-NE). Values are mean ± SE. Asterisk indicates significantly greater platelets in NE-inoculated animals with $p < 0.001$ by analysis of variance.

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